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Protein–quinone interactions in the bacterial photosynthetic reaction center: light-induced FTIR difference spectroscopy of the quinone vibrations

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1. Introduction

Quinones are ubiquitous redox components that are involved in a number of essential bioenergetic processes such as respiration and photosynthesis. Notably, they play a key role in the transmembrane electron and proton transfer reactions that take place in cytochrome bc_1, cytochrome b_6f, and in the photosynthetic reaction center (RC) of purple bacteria, cyanobacteria, algae, and plants. Although the redox properties of the quinones depend for a large part on their chemical nature, it is also clear that the interactions between the quinone and the protein at a specific binding site can further modulate the electronic properties and thus the redox potential of this cofactor in situ.

There are only a few biophysical techniques that allow an investigation of the details of the quinone–protein interactions in RCs and each of them has intrinsic limitations. X-ray crystallography [1–6], although most useful in providing essential information on the general geometry of the binding site and on the identity of the amino-acid residues lining this site, is somewhat limited in resolution so that some of the most interesting details (conformation of the ring and of the substituents, hydrogen bonding...) are lost in the blur of the atom positions. Furthermore, structures for only the resting state of the RC protein have been obtained so far. EPR and solid-state NMR techniques are currently used, but again only a single redox state of the system can be scrutinized. In contrast FTIR difference spectroscopy provides a mean to investigate both the oxidized and reduced states of these complexes and affords the selectivity and sensitivity to monitor even minute changes occurring at the level of individual bonds upon change of redox state of the enzyme. One of the main limitation of this technique, however, is that vibrational modes from all the bonds from both the cofactor and the protein that have been perturbed by a given redox transition are due to contribute, leading to crowded difference spectra. This makes it difficult to assign the quinone vibrations and to separate them from the overlapping protein modes. There is, however, a very powerful approach to achieve this goal whenever the cofactor under investigation can be extracted and reconstituted in the protein, namely, the replacement of this cofactor by chemically modified or isotopically labeled compounds. For general reviews in the application of FTIR techniques to photosynthetic reactions, the reader is referred to [7–9]. On the other hand, we feel that the discussion of the FTIR data in a recent and more general review on radicals in phothosynthetic systems [10] is most incomplete and often biased.

This contribution is intended to provide a brief overview of our present understanding of the quinone–protein interactions in photosynthetic reaction centers as derived from light-induced FTIR difference spectroscopy as well as a discussion of the results in the light of the available X-ray data.

Abbreviations: RC, reaction center; P, primary electron donor; Q_A, Q_B, primary, secondary quinone acceptor; *Rb.*, *Rhodobacter*; *Rp.*, *Rhodopseudomonas*; FTIR, Fourier transform infrared; Q_n, 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone; Vitamin K_1, 2-methyl-3-phytyl-1,4-naphthoquinone.

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2. The Q^-/Q FTIR difference spectra

The vibrational spectrum of the quinones Q_A and Q_B in their different states of ionization and/or protonation in photosynthetic RCs is expected to shed light on the geometrical and energetic factors (hydrogen bonding, dipolar interactions, distortion of the ring and substituents, microconformational changes, and electrostatic relaxation of the protein) involved in the charge separation and stabilization processes and in the protonation events. Since 1990, light-induced FTIR difference spectroscopy of various specific reactions involving the quinones has thus been implemented. The $P^+Q_A^-/PQ_A$ and $P^+Q_B^-/PQ_B$ difference spectra of RCs of *Rb. sphaeroides* and *Rp. viridis* have been analyzed [11–14]. It was soon realized, however, notably through the use of RCs reconstituted with chemically modified or isotopically labeled quinones [11], that the dominating contribution from P^+/P in these spectra tends to swamp out the vibrations associated with quinone reduction. Time-resolved FTIR (rapid-scan) techniques led to the first $Q_A^-Q_B/Q_AQ_B^-$ double-difference spectra of *Rb. sphaeroides* and *Rp. viridis* [15–17] and were complemented by direct kinetic measurements of Q_A^- to Q_B electron-transfer reactions [18,19]. In addition, various electrochemical conditions have been experimented to generate Q^-/Q FTIR difference spectra both in vitro [20–22] and in vivo [23–25].

Using a different approach, it has been demonstrated that pure Q_A^-/Q_A and Q_B^-/Q_B spectra free from contribution of other radicals could be directly obtained with a high signal-to-noise ratio by illuminating RCs or chromatophores in the presence of a reductant and a mediator that rapidly reduce P^+. Under these conditions, the reduced quinone and the associated proteic changes are the only detectable species that photoaccumulate. Using this method, pure Q_A^-/Q_A [22,26–28] and Q_B^-/Q_B spectra [29] have been characterized for both *Rb. sphaeroides* and *Rp. viridis* and have been compared to the available Q^-/Q FTIR difference spectra of the quinones in solution [20–22]. Comparison of the Q_A^-/Q_A spectrum of *Rp. viridis* RCs with that of other menaquinone-containing photosynthetic bacteria such as *Chromatium vinosum* and *Chloroflexus aurantiacus* revealed a common band pattern of three positive bands (at 1478, $\approx$ 1440, and $\approx$ 1390 cm^{-1}) assigned to the semiquinone vibrations [22].

The first pure Q_A^-/Q_A FTIR spectra were obtained by Berthomieu et al. on native PS II membranes [30] as well as after uniform ^{15}N labeling of the membranes [31]. The absence of contribution from other radical species was controlled by EPR measurements. A few tentative assignments were made, the clearest one being for an intense band at 1478 cm^{-1} attributed to the C⎓O mode of the semiquinone. The same observation and assignment were later reported by several authors [32–34]. On the other hand, another group contends that this band contains mainly contributions from the C⎓O mode of tyrosine$_Z$ [35–37] or even from modes of oxidized chlorophyll [38]. A more extensive discussion of the assignment of the 1478 cm^{-1} band to a mode of the Q_A^- plastosemiquinone radical in the Q_A^-/Q_A spectra [30,31] will be presented elsewhere (Hienerwadel et al., in preparation).

3. IR vibrations of the primary quinone acceptor (Q_A)

Once pure Q_A^-/Q_A spectra at high signal-to-noise ratio were obtained for the RC of purple bacteria [22,26–28], it became important to demonstrate that the classical Q_A extraction/reconstitution procedure [39] does not perturb the quinone vibrations [27]. This protocol was then used to

Table 1
Proposed assignment of the vibrational modes (cm^{-1}) of quinones in solution and in the Q_A and Q_B sites of bacterial reaction centers

	C=O	C=C	C⎓C	C⎓O	C⎓C
Vitamin K_1					
in solution	1661	1618 (quin.)			
		1597 (arom.)			
Q_A, *Rb. sphaeroides*	1651	1608 (quin.)	1478	1444	1394
	1640	1588 (arom.)			1388
Q_A, *Rp. viridis*	1653				
	1636	1585 (arom.)	1478	1438	1392
Ubiquinone					
in solution	1663	1611			
	1650				
Q_A, *Rb. sphaeroides*	1660 (C_1=O, C_{2in})	1628	1484	1466 (C_1⎓O)	1420
	1601 (C_4=O, C_{3out})			1448 (C_4⎓O)	
Q_B, *Rb. sphaeroides*	1641 (C_1=O, C_{2out})	$\approx$ 1615	$\approx$ 1490	1479	
	1641 (C_4=O, C_{3out})				
Q_B, *Rp. viridis*	1641 (C_1=O, C_{2out})	$\approx$ 1615	$\approx$ 1490	1475	
	1641 (C_4=O, C_{3out})				

quin., quinonic; arom., aromatic; C_{2in}, O–CH_3 bond of methoxy at C_2 in the quinone ring plane; C_{3out}, O–CH_3 bond of methoxy at C_3 out of the quinone ring plane.

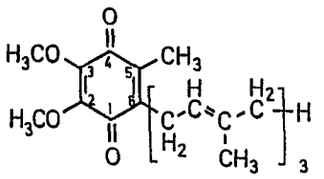

Fig. 1. Structural formula of ubiquinone-3 (Q_3).

reconstitute Q_A-depleted *Rb. sphaeroides* RCs with either a series of naphthoquinones bearing a variable number (0, 1, or 2) of methyl substituents or with vitamin K_1, which is a close analogue of the native menaquinone occupying the Q_A site of *Rp. viridis*. Further comparison with the spectra of *Rp. viridis* has permitted to propose assignment for some bands of the neutral Q_A with the main C=C mode at 1585 cm^{-1} and a C=O mode at 1636 cm^{-1} in *Rp. viridis* [27].

The first determination of the quinone vibrations in the Q_A^-/Q_A spectra of *Rb. sphaeroides* RCs was achieved by reconstituting Q_A – depleted RCs with isotopically labeled ubiquinones (Q_n, see Fig. 1) and vitamin K_1 [40]. The labels used were ^{18}O on both carbonyls (Q_1, Q_6, and vitamin K_1), ^{13}C uniformly (Q_8), and the combination of both labels (Q_8). Calculation of the isotopically labeled-minus-unlabeled double-difference spectra, which wipes out from the direct Q_A^-/Q_A difference spectra all the protein modes (as well as the quinone modes that are not affected by the label), led to the identification of most of the quinone bands (Table 1). The isotopic shifts observed on these bands were compared to those observed on the isolated quinone molecules and were used to propose preliminary assignments for the modes of both Q_A and Q_A^- [40]. Notably, a splitting of the carbonyl mode of vitamin K_1 in the Q_A site, giving rise to bands at 1651 and 1640 cm^{-1}, was observed. The frequency downshifts upon binding to the protein are 10 and 21 cm^{-1} (Table 1). Comparable downshifts have also been reported for the carbonyls of vitamin K_1 in the Q_A site of *Rp. viridis* [40] indicating similar binding of these menaquinones in the RCs of the two species (Table 1). For ubiquinone in the Q_A site of *Rb. sphaeroides*, a C=O vibration was found at 1660 cm^{-1}, essentially unperturbed compared to solution, while two modes of strongly mixed C=O and C=C character were detected at 1628 and 1601 cm^{-1}. Using ubiquinone (Q_3) selectively labeled with ^{13}C at either C_1 or C_4, it became possible to propose a more firm assignment for these modes of mixed character. Notably, the isotopic shifts are best interpreted in terms of a strong perturbation of the C_4=O carbonyl leading to a predominantly C_4=O mode at 1601 cm^{-1} and a predominantly C=C mode at 1628 cm^{-1} [41]. The proposed assignments for Q_A and Q_A^- are summarized in Table 1. While for the Q_A state, they are essentially identical to those proposed in a parallel study on *Rb. sphaeroides* [42], our assignments for the Q_A^- semiquinone vibrations differ significantly from those in [42]. However, our proposed ordering of the C⎓O and C⎓C modes of Q_A^- (Table 1) is consistent with the observation that upon $^1H/^2H$ exchange only one anion band in the Q_A^-/Q_A spectra is shifted [43]. This band is located at 1448 or at 1444 cm^{-1} in *Rb. sphaeroides* depending whether the RCs contain native ubiquinone or vitamin K_1, respectively, while in *Rp. viridis* it is found at 1438 cm^{-1} [43]. Such a shift upon $^1H/^2H$ exchange is more indicative of the perturbation of a hydrogen bonded C⎓O mode than of a C⎓C mode. Furthermore, one can observe that the ~ 1486 cm^{-1} band is the only anion mode that shifts upon $^{13}C_5$-labeling (using our numbering scheme, see Fig. 1) of Q_{10} in *Rb. sphaeroides* [42]. This shift appears more consistent with our assignment of the 1484 cm^{-1} band to a C⎓C mode (Table 1) than to a C⎓O mode. It should be stressed, however, that a more quantitative analysis of the inequivalence of the C_1⎓O and C_4⎓O modes will necessitate additional experimental data on the IR spectra of isotopically labeled semiquinones both in vivo and in vitro as well as a detailed normal mode analysis.

Our work with the native-like quinones has been complemented by a study of the vibrations of chainless symmetrical quinones reconstituted in the Q_A site of *Rb. sphaeroides* [44]. ^{18}O-labeling was used to discriminate the quinone modes from those of the protein. For menaquinones, the replacement of the chain by a methyl group has almost no influence on the bonding interactions with the protein. A quite different behavior is found with ubiquinones as the replacement of the chain by a methyl group perturbs appreciably the carbonyl vibrations. Since for ubiquinones the Q_A^-/Q_A spectra and the isotopic effects are essentially independent of the length of the isoprenyl chain as long as there is at least one isoprene unit [40], it was concluded that one or more of the last four carbon atoms of the first isoprene unit plays an important role in the proper anchoring of ubiquinone to the Q_A site [44].

These IR studies have also led to new informations on the conformation of the methoxy groups of ubiquinones. Firstly, the presence of two carbonyl bands in the IR spectra of these molecules in solution (Table 1) can be readily explained by the inequivalence of the conformation of the two adjacent methoxy groups (Fig. 1), with the high-energy C=O band corresponding to the mode of the carbonyl proximal to a methoxy group whose O-CH_3 bond lies in the quinone ring plane. Conversely, the low-energy band corresponds to the C=O mode for the carbonyl proximal to a methoxy with the O-CH_3 bond out of the quinone ring plane ([41], [44–47], and Burie et al., in preparation). On the other hand, the substituents at C_5 and C_6 have essentially no influence on the conformation of the methoxy groups although they can have some inductive effect on the proximal carbonyl. Thus, for ubiquinone (Q_1 to Q_{10}) in solution, a given carbonyl, e.g., C_1=O, has 50% of the proximal methoxy (at C_2) with the O–CH_3

bond in the quinone plane (C_1=O mode at 1663 cm^{-1}) and 50% of the proximal methoxy (at C_2) with the O–CH_3 bond out of this plane (C_1=O mode at 1650 cm^{-1}). The exact same situation occurs for C_4=O and the methoxy group at C_3. An IR marker band at 1263 cm^{-1} has been assigned to vibrations involving methoxy groups of the neutral quinone. In the Q_A site of *Rb. sphaeroides* this band is essentially the same as for ubiquinones in solution [40]. Furthermore by using ubiquinone selectively labeled at either carbonyl it has been proposed that in the Q_A site, all the population of the methoxy group at C_2 is preferentially oriented in the quinone plane, while all that at C_3 is more out of the Q_A plane [41,47]. If there were no other perturbation of the C_4=O carbonyl by interaction with the protein, a band at 1650 cm^{-1} would thus be expected instead of the 1601 cm^{-1} band observed in vivo.

The 49 cm^{-1} frequency downshift of the C_4=O carbonyl upon binding to the Q_A site of *Rb. sphaeroides* suggests a very strong hydrogen bond of the order of − 6 kcal mol^{-1}. Such a strong hydrogen bonding is often achieved in conjugated chelation systems when resonance involving delocalized charges comes into play to enhance the strength of the hydrogen bond. The assignment of the 1601 cm^{-1} band to a strongly hydrogen bonded carbonyl of Q_A agrees with the binding affinity studies of Gunner and Coll. [48,49] who have inferred the presence of only one strong hydrogen bond between the carbonyls of various quinones and the Q_A site with a binding free energy of −3 to − 7 kcal mol^{-1}. The 21 and 10 cm^{-1} downshifts experienced by the two carbonyls of vitamin K_1 upon binding to the Q_A site of *Rb. sphaeroides* (in *Rp. viridis* these shifts are 25 and 8 cm^{-1}, respectively) suggest weaker and less asymmetrical bonding interactions of the carbonyls of this menaquinone than in the case of ubiquinone.

4. IR vibrations of the secondary quinone acceptor (Q_B)

The first attempt at identifying the Q_B vibrations has been to compare Q_B^-/Q_B FTIR difference spectra of RCs reconstituted either with Q_{10} or with Q_0 [29]. This comparison was made for both *Rb. sphaeroides* and *Rp. viridis* giving additional insights in the binding of Q_B and Q_B^-. Several assignments could be proposed, notably a C=O mode at 1640 cm^{-1} and a C=C mode at 1616–1618 cm^{-1} for Q_{10} in the Q_B sites of both species as well as a C=O mode at 1479 and 1475 cm^{-1} in *Rb. sphaeroides* and *Rp. viridis*, respectively.

More definitive assignments came from the investigation of the Q_B^-/Q_B FTIR difference spectra of RCs of both species reconstituted with isotopically labeled ubiquinones. The labels used were ^{18}O on both carbonyls and ^{13}C either uniformly or at the 1- or the 4-position [47]. This study has demonstrated that the C_1 and C_4 carbonyls of Q_B contribute equally to the band at 1641 cm^{-1} and that the overall strength of the interaction that each carbonyl of Q_B is engaging with the protein at the binding site is weak and is equivalent for the C_1=O and the C_4=O groups (Table 1). Similar conclusions were derived from a parallel study of Q_{10} in *Rb. sphaeroides* by Brudler et al. [50] who also claimed the presence of an additional population of Q_B essentially free from interaction with the protein. While in our hands each of the C_1 and C_4 carbonyls of Q_B is perturbed to the very same extent upon binding to the protein, the C=C vibrations of Q_B exhibit a different behavior which is revealed upon selective ^{13}C labeling of the carbonyls. The C=C vibrations are decidedly not equivalent for selective labeling at C_1 or at C_4 although they both appear to contribute to the ~ 1615 cm^{-1} band in the Q_B^-/Q_B spectra. The comparison of the isotopic shifts observed in vivo and in vitro [47] shows that the protein at the Q_B site imparts a specific configuration to the quinone so that the C_4 atom is specifically perturbed compared to the isolated molecule. In contrast, the C_1 atom of Q_B behaves as it does in vitro or in the Q_A site of *Rb. sphaeroides*.

The reasons for this clear differential effect of the protein on C_1 and C_4 in the Q_B site while the vibrational frequency of the two C=O groups is the same are still obscure. It is striking, however, that in the Q_A site of *Rb. sphaeroides* the C_4 atom of the ubiquinone is also dramatically perturbed, while the C_1 atom behaves essentially as in the isolated molecule. While the strong hydrogen bond at the C_4 carbonyl of Q_A has been taken as responsible for the large effect on the C=C vibrations coupled to the C_4=O mode [41], this cannot be the reason for the differential effect seen on the C=C band of Q_B. In order to explain this effect of the protein on the differential coupling of the C=C mode of Q_B to vibrations involving displacements of the C_1 and C_4 atoms, another possibility is to invoke the interactions of the peripheral substituents with the protein at the binding site. Indeed, it can be surmised that a specific anchoring of the 5'-methyl and/or of the methoxy group at C_3 into a tight binding niche could perturb the C=C mode coupled to the C_4=O mode much more than that coupled to the C_1=O mode provided the chain at C_6 and the methoxy group at C_2 are less distorted. Our FTIR data have provided some evidence that within the Q_B site the substituents at C_3 and C_5 are in a more constrained environment than those at C_2 and C_6. Notably, it has been suggested that both methoxy groups of the neutral Q_B have their O–CH3 bond out of the quinone ring plane [47]. Semi-empirical calculations have shown that the preferred geometry of the semiubiquinone in solution has the two methoxy groups out of the quinone plane ([45] and Burie et al., in preparation). Thus, the difference in the conformation of the methoxy groups of ubiquinone in the Q_A and Q_B sites provides a rational for the higher redox potential of Q_B compared to Q_A. Additional information on the conformation of the methoxy

groups of Q_A and Q_B derived from FTIR difference spectroscopy using Q_3 selectively labeled at C_2 or at C_3 will be presented elsewhere (Breton et al., in preparation).

When the vibrations of Q_B in the RCs of *Rb. sphaeroides* are compared to those in *Rp. viridis*, the observation of an almost identical frequency and of an identical extent of coupling for each of the C=O and C=C strongly suggests identical interactions in both RCs. More generally, the remarkably close similarity in the pattern and the relative amplitudes of the positive and negative bands observed in the double-difference spectra for the isotopic effects on the Q_B vibrations in the two species [47,51] points to a close correspondence of the Q_B and Q_B^- vibrations and thus a close equivalence of the geometry of the ubiquinone at the Q_B site in the RCs of *Rb. sphaeroides* and *Rp. viridis* in both the neutral and reduced states.

5. Comparison of the FTIR data with crystallographic structures

The bonding interactions of Q_A with the protein are relatively well defined (within the ± 0.25–0.5 Å precision on the position of the non-hydrogen atoms in the X-ray data) although the details vary among the various structures [1–6], notably with respect to the identity of the proton-donating residues involved in hydrogen bonding of the two carbonyls and in the relative strength of the two hydrogen bonds. The C_1=O group corresponds to that proximal to the isoprenoid chain which, according to the description of the X-ray structures at the Q_A site of *Rb. sphaeroides*, is the carbonyl group in strong hydrogen bonding interaction with the peptide NH of Ala-M260 (donor–acceptor distance 2.5–2.8 Å). The C_4=O group is proposed to interact more weakly with either Thr-M222 [2,5,6] or His-M219 [3]. In the highest resolution X-ray structure available for *Rb. sphaeroides* [3], the C_4 carbonyl is positioned in hydrogen bonding distance (3.2 Å) with the proton (at Nδ1) of the His-M219 residue which is also ligand (at Nϵ2) to the non-heme Fe^{2+} atom. A comparable positioning of the carbonyls of the menaquinone is also proposed for the Q_A site of *Rp. viridis* with a distance of 3.1 Å for both hydrogen bonds [4].

In view of the X-ray structures, it has been suggested that the dramatic downshift of the frequency of the C_4 carbonyl of Q_A may reflect the formation of a specific hydrogen bond at the Nδ1 atom of the side chain of His-M219 (M217 in *Rp. viridis*) stabilized by resonance interaction to the Fe^{2+} atom via the imidazole ring system [41]. On the other hand, the C_1=O carbonyl of Q_A appears not to interact with the protein. This last observation is thus in sharp contrast with all the X-ray data available on RCs which have been interpreted in terms of a strong hydrogen bond between C_1=O and the peptide N–H of Ala-M260 (M258 in *Rp. viridis*).

The description of the binding site of Q_B differs wildly for the various structural models. In a recent study the X-ray structures of the different RCs were overlaid to show that from one structure to the other, the position of the center of Q_B varies by ~ 5 Å [4]. This variability in the position of Q_B may reflect the function of this acceptor which, upon double reduction, leaves the protein so that it must be located near a conduct to the exterior. To make this situation even worse, the two extreme locations of Q_B are found in the two highest resolution structures presently available, one being for *Rb. sphaeroides* [3] and the other for *Rp. viridis* [4]. In the best available X-ray structure of the Q_B site [4], the carbonyl oxygen at C_1 is proposed to accept two hydrogen bonds from the peptide nitrogen of Ile-L224 (at a distance of 3.1 Å) and Gly-L225 (distance 3.0 Å) while the C_4 carbonyl accepts a hydrogen bond from the Nδ1 atom of His-L190 (distance 2.7 Å), which is also a ligand (at Nϵ2) to the non-heme Fe^{2+}. This pattern of interactions of His-L190 with both the non-heme Fe^{2+} and the C_4=O group of Q_B in *Rp. viridis* is thus very close to that proposed for His-M219 with the non-heme Fe^{2+} and the C_4=O group of Q_A in *Rb. sphaeroides* [3]. Owing to the pseudo-twofold symmetry of the RCs, the overall orientation of the hydrogen bond partners is also conserved at the Q_A and Q_B sites.

The finding that the frequency of the C_4=O group is much less perturbed for Q_B than for Q_A shows that electronic coupling of the C_4=O of Q_A with the non-heme Fe^{2+} atom through the imidazole ring of His-M219 is probably not the main reason for the strong downshift of the C_4=O mode of Q_A. Taking into account the evidence for identical bonding interactions of Q_B in the RC of both species derived from our FTIR data, the highest resolution X-ray results for Q_A [3] and Q_B [4] imply a shorter hydrogen bond length at the C_4=O of Q_B (2.7 Å) than at the C_4=O of Q_A (3.2 Å) in *Rb. sphaeroides*. Thus, the best X-ray structures for the quinone binding sites in RCs give no clues to the large differences in the vibrational frequency of the C_4 carbonyl of Q_A and Q_B detected by light-induced FTIR difference spectroscopy in *Rb. sphaeroides*. It is particularly striking that the C_4=O mode of Q_A experiences upon binding a much larger downshift (49 cm^{-1}) than that of Q_B (9 cm^{-1}) while, considering the present X-ray structures, one would have made the opposite prediction.

Compared to the large variations in the proposed bonding interactions of Q_B derived from the interpretation of the X-ray data [1–6], the remarkable equivalence of the C=O and C=C vibrations of Q_B in *Rb. sphaeroides* and *Rp. viridis* unambiguously demonstrates the almost identical bonding pattern and conformation of the neutral Q_B in the RCs of the two species. Taking the newest structure of *Rp. viridis* [4] as the most reliable representation presently available of the Q_B pocket in bacterial RC, it can thus be stated that it also provides the description of the native Q_B site in the RCs of *Rb. sphaeroides*. In our opinion, higher

resolution structures should further confirm the close identity of the Q_B conformation in the RCs of both species.

6. Conclusions and prospects

Taken together with the data from X-ray crystallography, light-induced FTIR difference spectroscopy of photosynthetic reactions can provide unique information on the bonding interactions of the cofactors involved in the reactions and on the structural changes occurring in both the cofactors and the protein. Whenever this technique can be combined with selective isotope labeling of one cofactor, as for example in the case of the quinones in bacterial RCs described in this contribution, individual modes can be assigned. In turns the FTIR data complement the structural information by providing new details that were not always accessible to X-ray crystallography techniques. Even when apparent discrepancies appear in the conclusions derived from both techniques, this complementarity should be further utilized to resolve the issue.

Regarding the quinones in RCs, a number of unresolved questions await further FTIR experiments and several of them are currently investigated in our group. The geometry of the methoxy groups of ubiquinone in the Q_A and Q_B sites is being probed with ubiquinones selectively labeled at C_2 or at C_3. The two carbonyl groups of menaquinone in the Q_A site of *Rp. viridis* and of *Rb. sphaeroides* are being identified using vitamin K_1 selectively labeled on either one of the carbonyls. The influence of the peripheral substituents on the Q_B^-/Q_B of *Rp. viridis* is being studied by reconstituting the RCs with Q_B analogues that are both chemically modified and isotopically labeled. Finally, with a number of the quinone modes now being assigned, we are currently investigating the response of the protein and of the other cofactors upon quinone reduction. This approach combines site-directed mutagenesis [52–54], $^1H/^2H$ exchange [52,54], and selective labeling of a single type of amino-acid residue.

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References

[1] Deisenhofer, J. and Michel, H. (1989) EMBO J. 8, 2149–2169.
[2] Feher, G., Allen, J.P., Okamura, M.Y. and Rees, D.C. (1989) Nature 339, 111–116.
[3] Ermler, U., Fritzsch, G., Buchanan, S.K. and Michel, H. (1994) Structure 2, 925–936.
[4] Lancaster, C.R.D., Ermler, U. and Michel, H. (1995) in Anoxygenic Photosynthetic Bacteria (Blankenship, R.E., Madigan, M.T. and Bauer, C.E., eds.), pp. 503–526, Kluwer Academic, Dordrecht.
[5] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1988) Proc. Natl. Acad. Sci. USA 85, 8487–8491.
[6] El-Kabbani, O., Chang, C.-H., Tiede, D., Norris, J. and Schiffer, M. (1991) Biochemistry 30, 5361–5369.
[7] Hoff, A.J. (1992) Isr. J. Chem. 32, 405–412.
[8] Mäntele, W. (1995) in Anoxygenic Photosynthetic Bacteria (Blankenship, R.E., Madigan, M.T. and Bauer, C.E., eds.), pp. 627–647, Kluwer, Dordrecht.
[9] Nabedryk, E. (1996) in Infrared Spectroscopy of Biomolecules (Mantsch, H.H. and Chapman, D., eds.), pp. 39–81, Wiley-Liss, New York.
[10] Angerhofer, A. and Bittl, R. (1996) Photochem. Photobiol. 63, 11–38.
[11] Bagley, K., Abresch, E., Okamura, M.Y., Feher, G., Bauscher, M., Mäntele, W., Nabedryk, E. and Breton, J. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 77–80, Kluwer Academic, Dordrecht.
[12] Buchanan, S., Michel, H. and Gerwert, K. (1990) in Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.-E., ed.), pp. 75–85, Springer, Berlin.
[13] Buchanan, S., Michel, H. and Gerwert, K. (1992) Biochemistry 31, 1314–1322.
[14] Nabedryk, E., Bagley, K., Thibodeau, D.L., Bauscher, M., Mäntele, W. and Breton, J. (1990) FEBS Lett. 266, 59–62.
[15] Thibodeau, D.L., Breton, J., Berthomieu, C., Bagley, K., Mäntele, W. and Nabedryk, E. (1990) in Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.-E., ed.), pp. 87–98, Springer, Berlin.
[16] Thibodeau, D.L., Nabedryk, E., Hienerwadel, R., Lenz, F., Mäntele, W. and Breton, J. (1990) Biochim. Biophys. Acta 1020, 253–259.
[17] Thibodeau, D.L., Nabedryk, E., Hienerwadel, R., Lenz, F., Mäntele, W. and Breton, J. (1992) in Time-Resolved Vibrational Spectroscopy V (Takahashi, H., ed.), pp. 79–82, Springer, Berlin.
[18] Hienerwadel, R., Thibodeau, D., Lenz, F., Nabedryk, E., Breton, J., Kreutz, W. and Mäntele, W. (1992) Biochemistry 31, 5799–5808.
[19] Hienerwadel, R., Nabedryk, E., Breton, J., Kreutz, W. and Mäntele, W. (1992) in The Photosynthetic Bacterial Reaction Center II: Structure, Spectroscopy, and Dynamics (Breton, J. and Verméglio, A., eds.), pp. 155–162, Plenum Press, New York.
[20] Bauscher, M., Nabedryk, E., Bagley, K., Breton, J. and Mäntele, W. (1990) FEBS Lett. 261, 191–195.
[21] Bauscher, M. and Mäntele, W. (1992) J. Phys. Chem. 96, 11101–11108.
[22] Breton, J., Bauscher, M., Berthomieu, C., Thibodeau, D.L. Andrianambinintsoa, S., Dejonghe, D., Mäntele, W. and Nabedryk, E. (1991) in Spectroscopy of Biological Molecules (Hester, R.E. and Girling, R.B., eds.), pp. 43–46, The Royal Society of Chemistry, Cambridge.
[23] Mäntele, W., Leonhard, M., Bauscher, M., Nabedryk, E., Breton, J. and Moss, D.A. (1990) in Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.-E., ed.), pp. 31–44, Springer, Berlin.
[24] Bauscher, M., Leonhard, M. Moss, D.A. and Mäntele, W. (1993) Biochim. Biophys. Acta 1183, 59–71.
[25] Bauscher, M., Mäntele, W. and Dutton, P.L. (1993) in: 5th Int. Conf. Spectroscopy of Biological Molecules (Theophanides, T., Anastassopoulou, J. and Fotopoulos, N., eds.), pp. 319–320, Kluwer, Dordrecht.
[26] Breton, J., Thibodeau, D.L., Berthomieu, C., Mäntele, W., Verméglio, A. and Nabedryk, E. (1991) FEBS Lett. 278, 257–260.
[27] Breton, J., Burie, J.-R., Berthomieu, C., Thibodeau, D.L. Andrianambinintsoa, S., Dejonghe, D., Berger, G. and Nabedryk, E. (1992) in The Photosynthetic Bacterial Reaction Center II: Structure,

Spectroscopy, and Dynamics (Breton, J. and Verméglio, A., eds.), pp. 155–162, Plenum Press, New York.
[28] Nabedryk, E., Berthomieu, C., Verméglio, A. and Breton, J. (1991) FEBS Lett. 293, 53–58.
[29] Breton, J., Berthomieu, C., Thibodeau, D.L. and Nabedryk, E. (1991) FEBS Lett. 288, 109–113.
[30] Berthomieu, C., Nabedryk, E., Mäntele, W. and Breton, J. (1990) FEBS Lett. 269, 363–367.
[31] Berthomieu, C., Nabedryk, E., Breton, J. and Boussac, A. (1992) in Research in Photosynthesis (Murata, N., ed.), Vol. II, pp. 53–56, Kluwer, Dordrecht, The Netherlands.
[32] Noguchi, T., Ono, T.-a. and Inoue, Y. (1992) Biochemistry 31, 5953–5956.
[33] Toyoshima, Y., Kitamura, K., Sasaki, J., Maeda, A., Araga, C. and Akabori, K. (1992) in Research in Photosynthesis (Murata, N., ed.), Vol. II, pp. 127–130, Kluwer, Dordrecht, The Netherlands.
[34] Araga, C., Akabori, K., Sasaki, J., Maeda, A., Shiina, T. and Toyoshima, K. (1993) Biochim. Biophys. Acta 1142, 36–42.
[35] MacDonald, G.M. and Barry, B.A. (1992) Biochemistry 31, 9848–9856.
[36] MacDonald, G.M., Bixby, K.A. and Barry, B.A. (1993) Proc. Natl. Acad. Sci. USA 90, 11024–11028.
[37] Bernard, M.T., MacDonald, G.M., Nguyen, A.P., Debus, R.J. and Barry, B.A. (1995) J. Biol. Chem. 270, 1589–1594.
[38] MacDonald, G.M., Steenhuis, J.J. and Barry, B.A. (1995) J. Biol. Chem. 270, 8420–8428.
[39] Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3491–3495.
[40] Breton, J., Burie, J.-R., Berthomieu, C., Berger, G. and Nabedryk, E. (1994) Biochemistry 33, 4953–4965.
[41] Breton, J., Boullais, C., Burie, J.-R., Nabedryk, E. and Mioskowski, C. (1994) Biochemistry 33, 14378–14386.
[42] Brudler, R., de Groot, H.J.M., van Liemt, W.B.S., Steggerda, W.F., Esmeijer, R., Gast, P., Hoff, A.J., Lugtenburg, J. and Gerwert, K. (1994) EMBO J. 13, 5523–5530.
[43] Breton, J. and Nabedryk, E. (1995) in Photosynthesis: from Light to Biosphere (Mathis, P., ed.), pp. 395–400, Kluwer, Dordrecht, The Netherlands.
[44] Breton, J., Burie, J.-R., Boullais, C., Berger, G. and Nabedryk, E. (1994) Biochemistry 33, 12405–12415.
[45] Burie, J.-R. (1994) Ph.D Thesis, University of Paris VI.
[46] Burie, J.-R., Boussac, A., Boullais, C., Berger, G., Mattioli, T., Mioskowski, C., Nabedryk, E. and Breton, J. (1995) J. Phys. Chem. 99, 4059–4070.
[47] Breton, J., Boullais, C., Mioskowski, C. and Nabedryk, E. (1995) Biochemistry 34, 11606–11616.
[48] Gunner, M.R., Braun, B.S., Bruce, J.M. and Dutton, P.L. (1985) in Antennas and Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.-E., ed.), pp. 298–305, Springer, Berlin.
[49] Warncke, K., Gunner, M.R., Braun, B.S., Gu, L., Yu, C.-A., Bruce, J.M. and Dutton, P.L. (1994) Biochemistry 33, 7830–7841.
[50] Brudler, R., De Groot, H.J.M., van Liemt, W.B.S., Gast, P., Hoff, A.J., Lugtenburg, J. and Gerwert, K. (1995) FEBS Lett. 370, 88–92
[51] Breton, J., Nabedryk, E., Mioskowski, C. and Boullais, C. (1995) in Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.-E., ed.), pp. 381–404, Springer, Berlin.
[52] Breton, J., Nabedryk, E., Allen, J.P. and Williams, J.C. (1996) Biophys. J. 70, A355.
[53] Hienerwadel, R., Grzybek, S., Fogel, C., Kreutz, W., Okamura, M.Y., Paddock, M.L., Breton, J., Nabedryk, E. and Mäntele, W. (1995) Biochemistry 34, 2832–2843.
[54] Nabedryk, E., Breton, J., Hienerwadel, R., Fogel, C., Mäntele, W., Paddock, M.L. and Okamura, M.Y. (1995) Biochemistry 34, 14722–14732.